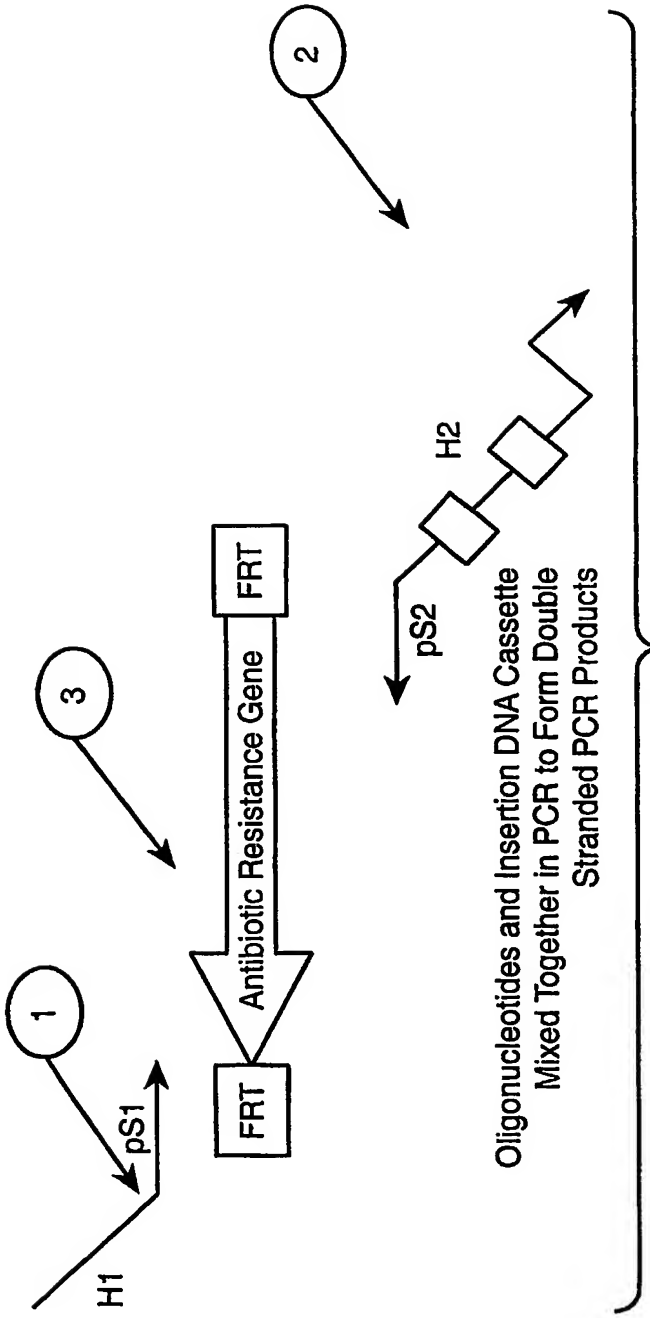
1
pS1
H1
3
FRT
Antibiotic Resistance Gene
FRT
2
H2
pS2
Oligonucleotides and Insertion DNA Cassette
Mixed Together in PCR to Form Double
Stranded PCR Products

FIG._1A

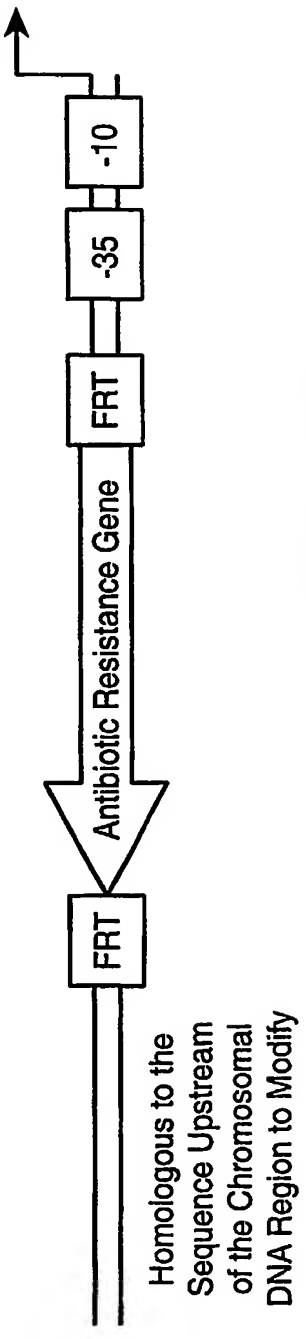
-10
-35
FRT
Antibiotic Resistance Gene
FRT
Homologous to the
Sequence Upstream
of the Chromosomal
DNA Region to Modify

FIG._1B

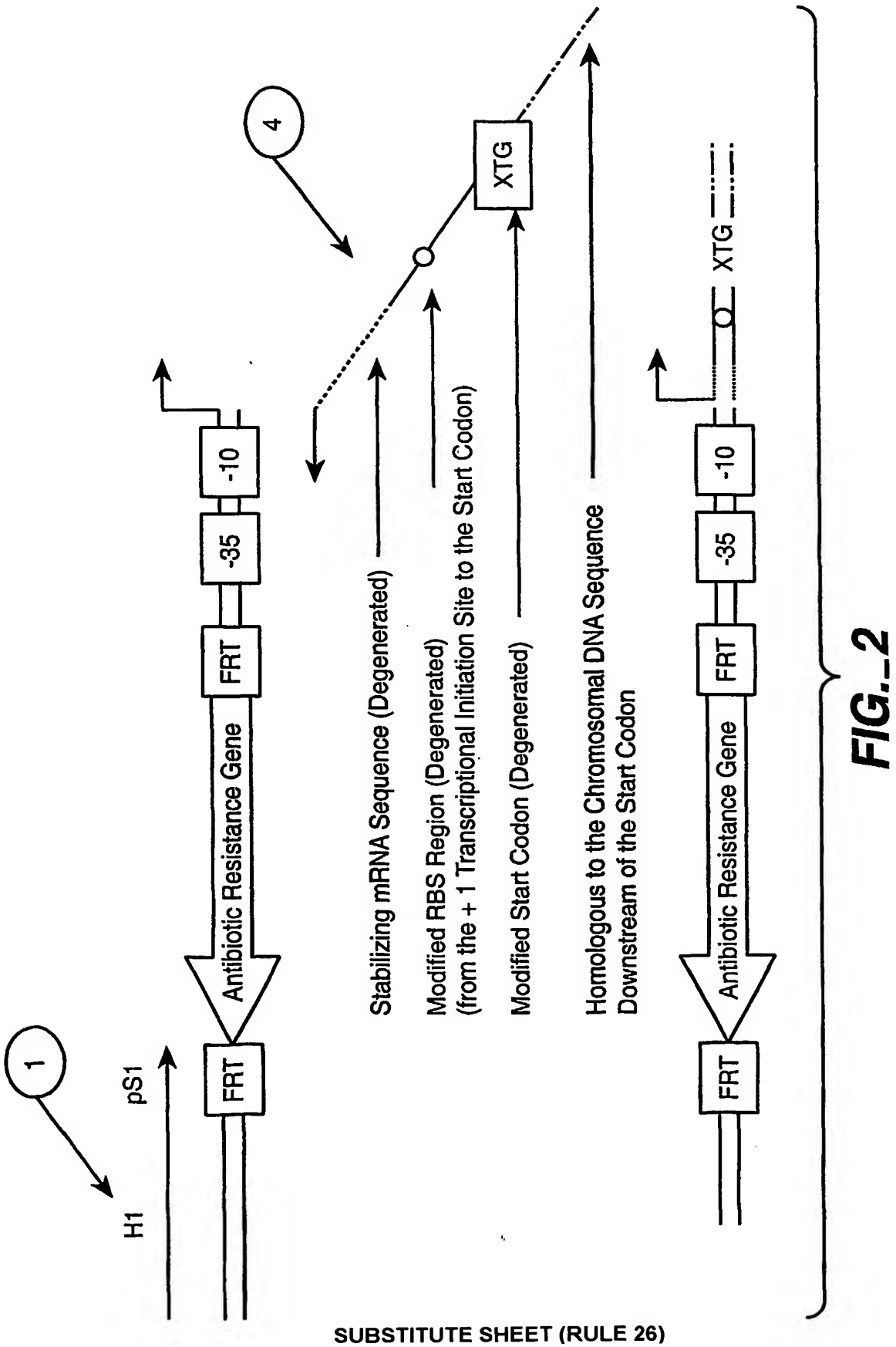
1
H1
pS1
FRT
Antibiotic Resistance Gene
FRT
-35
-10
4
XTG
Stabilizing mRNA Sequence (Degenerated)
Modified RBS Region (Degenerated)
(from the + 1 Transcriptional Initiation Site to the Start Codon)
Modified Start Codon (Degenerated)
Homologous to the Chromosomal DNA Sequence
Downstream of the Start Codon
XTG
-10
-35
FRT
Antibiotic Resistance Gene
FRT

FIG._2

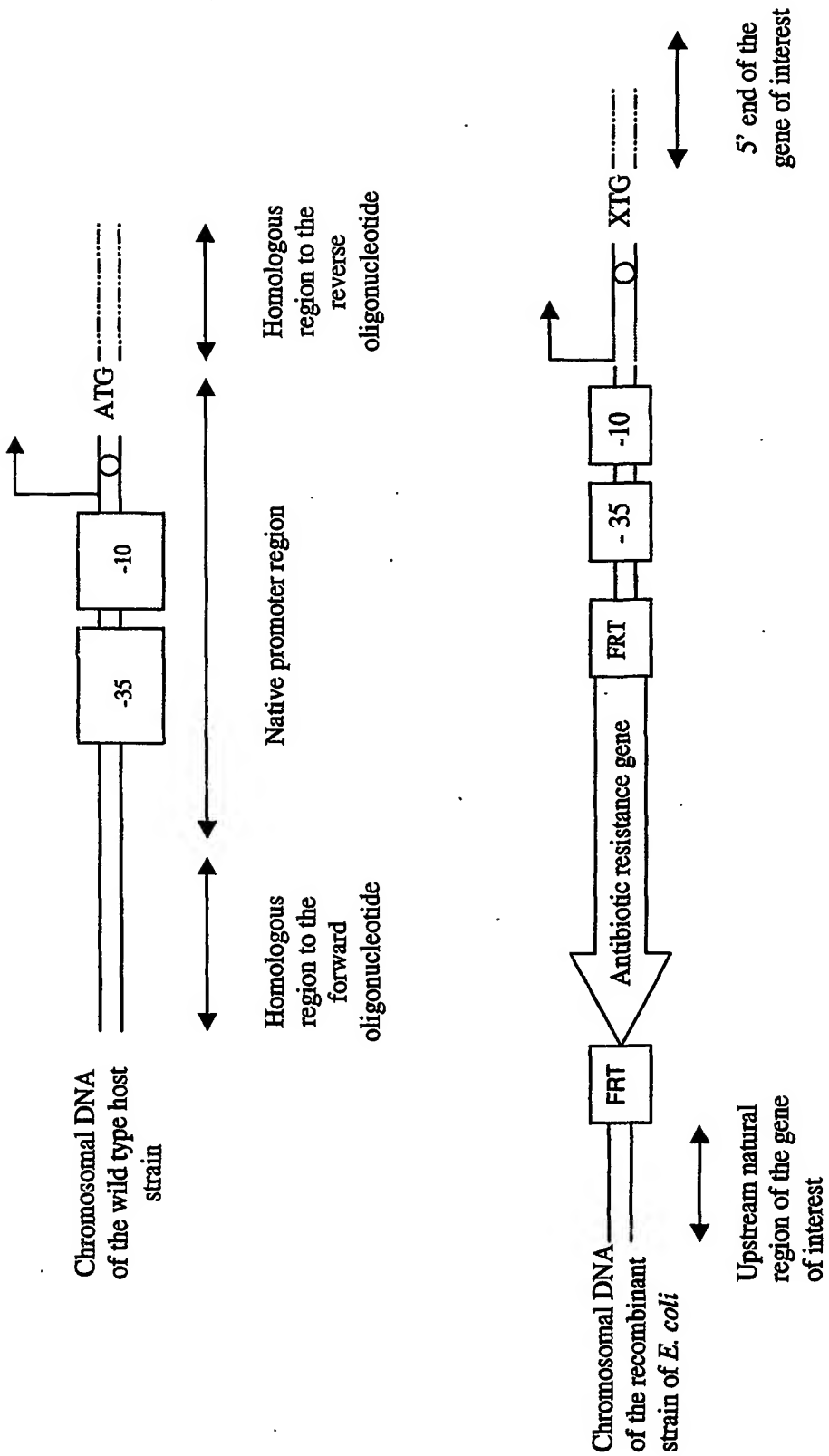
Chromosomal DNA of the wild type host strain
-35
-10
ATG
Homologous region to the forward oligonucleotide
Native promoter region
Homologous region to the reverse oligonucleotide
Chromosomal DNA of the recombinant strain of E. coli
FRT
Antibiotic resistance gene
FRT
- 35
-10
XTG
Upstream natural region of the gene of interest
5' end of the gene of interest

FIGURE 3

FIGURE 4

	-50		-7	+1	+20	
PH/E20	AACTGCAAAAATAGT	**TTGACA**	CCCTAGCCGATAGGCTT	**TAAGAT**	GTACCCAGTTCGATGAGAGCGATAAC	(SEQ ID NO. 3)
PH207	TTTTAAAAAATTCAT	**TTGCTA**	AACGCTTCAAATTCTCG	**TATAAT**	ATACTTCATAAATTGATAAACAAAAA	(SEQ ID NO. 4)
PN25	TCATAAAAAATTTAT	**TTGCTT**	TCAGGAAAATTTTTCTG	**TATAAT**	AGATTCATAAATTGAGAGAGGAGTT	(SEQ ID NO. 5)
PG25	TGAAAAATAAAATTC	**TTGATA**	AAATTTTCCAATACTAT	**TATAAT**	ATTGTTATTAAAGAGGAGAAATTAAC	(SEQ ID NO. 6)
PJ5	ATATAAAAACCGTTA	**TTGACA**	CAGGTGGAAATTTAGAA	**TATACT**	GTTAGTAAACCTAATGGATCGACCTT	(SEQ ID NO. 7)
PA1	TTATCAAAAAGAGTA	**TTGACT**	TAAAGTCTAACCTATAG	**GATACT**	TACAGCCATCGAGAGGGACACGGCGA	(SEQ ID NO. 8)
PA2	CACGAAAAACAGGTA	**TTGACA**	ACATGAAGTAACATGCAG	**TAAGAT**	ACAAATCGCTAGGTAACACTAGACGC	(SEQ ID NO. 9)
PA3	GGTGAAACAAAACGG	**TTGACA**	CACTGAAGTAAACACGG	**TACGAT**	GTACCACATGAAACGACAGTGAGTCA	(SEQ ID NO. 10)
PL	TTATCTCTGGCGGTG	**TTGACA**	TAAATACCACTGGCGGT	**GATACT**	GAGCACATCAGCAGGACGCACTGACC	(SEQ ID NO. 11)
Plac	TTAGGCACCCCAGGC	**TTTACA**	CTTTATGCTTCCGGCTGG	**TATGTT**	GTGTGGAATTGTGAGCGATAACAAT	(SEQ ID NO. 1)
PlacUV5	CTAGGCACCCCAGGC	**TTTACA**	CTTTATGCTTCCGGCTGG	**TATAAT**	GTGTGGAATTGTGAGCGGATAACAAT	(SEQ ID NO. 12)
PtacI	TTCTGAAATGAGCTG	**TTGACA**	ATTAATCATCGGCTCG	**TATAAT**	GTGTGGAATTGTGAGCGGATAACAAT	(SEQ ID NO. 2)
Pcon	AATTCACCGTCGTTG	**TTGACA**	TTTTTAAGCTTGGCGGT	**TATAAT**	GGTACCATAAGGAGGTGGATCCGGCA	(SEQ ID NO. 13)
Pbls	TTTTTTCTAAATACA	**TTCAAA**	TATGTATCCGCTCATGA	**GACAAT**	AACCCTGATAAATGCTTCAATAATAT	(SEQ ID NO. 14)

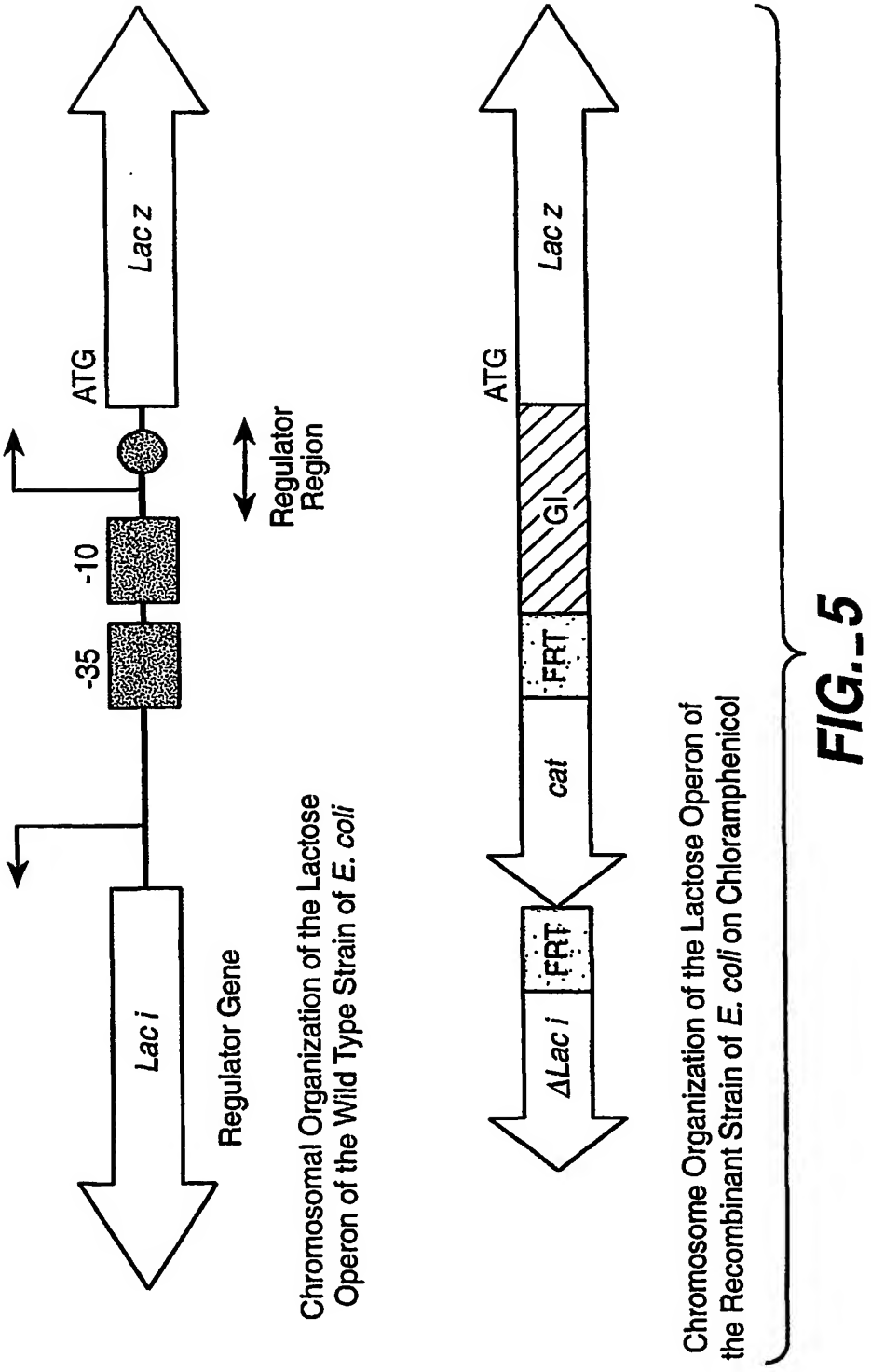
ATG
Lac z
Regulator Region
-10
-35
Lac i
Regulator Gene
Chromosomal Organization of the Lactose Operon of the Wild Type Strain of E. coli
ATG
Lac z
GI
FRT
cat
FRT
ΔLac i
Chromosome Organization of the Lactose Operon of the Recombinant Strain of E. coli on Chloramphenicol

FIG._5

FIGURE 6

pLAC (SEQ ID NO. 18)

AGGC TTTACA CTTTATGCTTCCGGCTCG TATGTT GTGTGGA ATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT ATGACC

- 35 **-10** **RBS** **Start**

1.6GI lacZ (SEQ ID NO. 19)

GCCC TTGACA ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT **ATGACC**

-35 **-10** **RBS** **start**

GI 1.6 (SEQ ID NO. 15)

GCCC **TTGACA** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

1.5GI lacZ (SEQ ID NO. 20)

GCCC TTGACT ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT **ATGACC**

-35 **-10** **RBS** **start**

GI 1.5 SEQ ID NO. 16

GCCC **TTGACT** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

1.20GI lacZ (SEQ ID NO. 21)

GCCC TTGACG ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT **ATGACC**

-35 **-10** **RBS** **start**

GI 1.2 (SEQ ID NO. 17)

GCCC **TTGACG** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

WO 03/089605 PCT/US03/12045

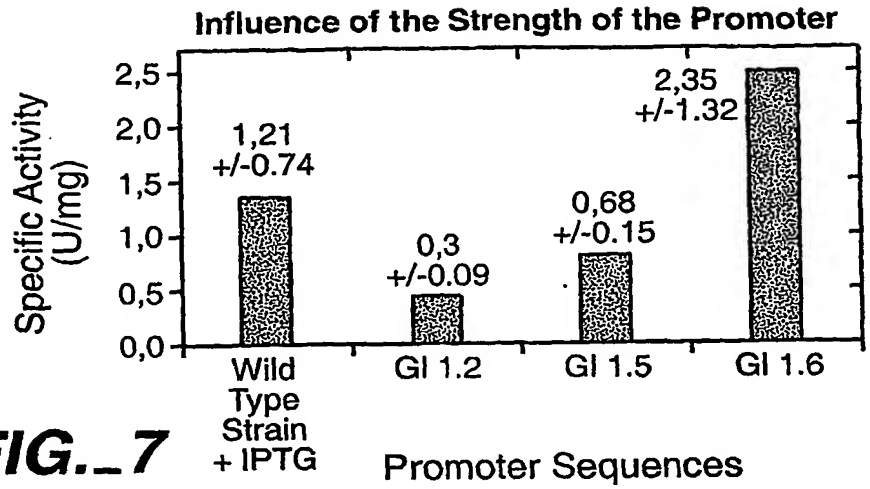

FIG._7

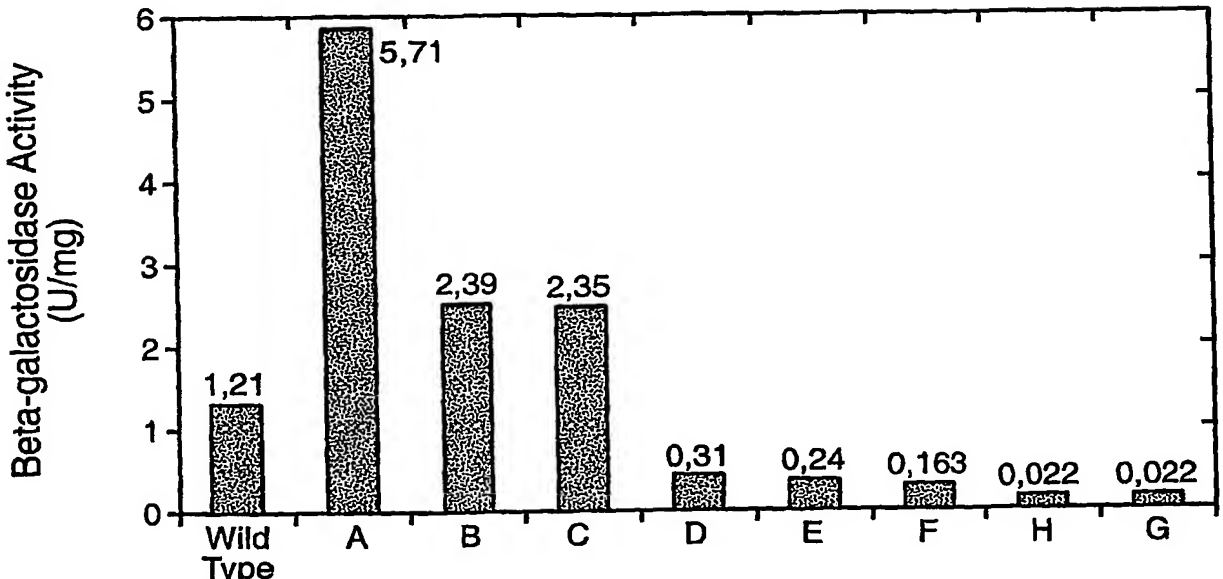

FIG._8

Transformants	Stabilizing Sequence	RBS Sequence	Specific Activity (U/mg)
mLac RNA 1	GGTCGAG	AAGGAGGAAA	5.71
mLac RNA 2	GGTGGAG	AAGGAGGAAA	11.04
mLac RNA 3	CCTCGAG	AAGGAGGAAA	18.44
mLac RNA 4	GGTGGAC	AAGGAGGAAA	7.3
mLac RNA 5	GCTGGAC	AAGGAGGAAA	4.11
Wild-type Strain +IPTG	NO	AGGAAA	1.21

FIG._9